Environ. Sci. Technol. **1998**, *32*, 1832−1840

Combined Laboratory/Field Study on the Use of Nitrate for in Situ Bioremediation of a Fuel-Contaminated Aquifer

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A pilot demonstration project was conducted at Eglin Air Force Base, FL, to compare the extent of bioremediation of a fuel-contaminated aquifer using sprinkler application with and without nitrate addition on two adjacent 30 m × 30 m cells. Target compound groups included both BTEXTMB (benzene, toluene, ethylbenzene, xylenes, and trimethylbenzenes) and the JP-4 jet fuel. Bioremediation performance was monitored through the use of groundwater quality measurements as well as periodic core analyses. Operation began April 1994, and an interim performance evaluation was conducted August 1994. The final performance evaluation was conducted May 1995. Minimal remediation occurred during the first four months. Water quality analyses showed that the nitrate cell subsurface was actively denitrifying, but lysimeter samples indicated that much of the nitrate was consumed within the rhizosphere above the fuel-contaminated interval. A 9 m × 9 m plot inside each cell was therefore stripped of vegetative cover and covered with a weed barrier to enhance nitrate transfer into the subsurface. After an additional 8 months operation, lysimeter samples showed increased nitrate transfer to the contaminated interval beneath the nitrate cell stripped plot, and there was higher fractional removal of contaminant groups beneath the stripped plots as well. On the basis of core data, BTEXTMB was reduced by 66 ± 1% in both treatment cells, equivalent to a mass loss of 106 and 21 kg in the nitrate cell and control cell, respectively. Monitoring well data provided evidence of sulfate reduction in the control cell but not in the nitrate cell. In addition, post-test treatability studies using core material from both cells demonstrated removal of alkylbenzenes under denitrifying and iron-reducing conditions, with different profiles for each cell. One year after completion of the project, BTEXTMB reductions in downgradient monitoring wells remained consistent with the core data. Collective laboratory and field data indicated that contaminant reduction occurred as a result of anaerobic bioremediation as well as soil washing and that different anaerobic processes predominated in the control cell due to circulation of endogenous electron acceptors.

Introduction

Leaking underground storage tanks are a major source of groundwater contamination by petroleum hydrocarbons. There have been 318 000 confirmed releases as of October 1996, and the EPA expects another 100 000 confirmed releases over the next several years (*1*). Gasoline and other fuels contain benzene, toluene, ethylbenzene, and xylenes (collectively known as BTEX), which are hazardous compounds regulated by the U.S. Environmental Protection Agency (*2*). In many cases, the problem is mitigated through the use of in situ aerobic bioremediation, which involves the addition of nutrients and oxygen (or hydrogen peroxide) to the contaminated areas so that the indigenous microbial populations can degrade the contaminants (*3*−*5*). Although aerobic bioremediation has been successfully applied (*6*−*8*), difficulties relating to aquifer plugging and oxygen mass transport are often encountered when water containing oxygen or hydrogen peroxide is introduced into anaerobic subsurface environments (*9*−*11*). This does not appear to be as much of a problem in bioventing or biosparging operations where air is used as the circulating medium instead of water (*12, 13*). However, these technologies are inappropriate where shallow water tables are encountered (*14, 15*) and can be ineffective where contaminants are trapped within the interior of the soil matrix (*16*).

Nitrate can also serve as an electron acceptor and results in anaerobic biodegradation of organic compounds via the processes of nitrate reduction and denitrification (*17*). Because nitrate is less expensive and more soluble than oxygen, it may be more economical to remediate fuel-contaminated aquifers using nitrate rather than oxygen. Several investigators have demonstrated that monoaromatic hydrocarbons, with the possible exception of benzene, can be degraded under denitrifying conditions (*18*−*25*). This holds true for other fuel constituents, such as polycyclic aromatic hydrocarbons (*26*−*28*) and breakdown products (*29*−*36*).

Several field studies have been performed on nitrate-based bioremediation of fuel-contaminated aquifers. Results include complete removal of benzene and toluene with the xylenes being more recalcitrant (*37*), a 95−98% reduction in purgeable alkylbenzenes (*38*); complete removal of toluene with benzene, ethylbenzene, and the xylenes being unaffected (*39*); and reductions of 87%, 67%, and 34% for toluene, ethylbenzene, and xylenes, respectively, with benzene being recalcitrant (*40*). Hutchins et al. (*41*) investigated the use of nitrate to promote biological removal of fuel aromatic hydrocarbons from a JP-4 jet fuel spill at Traverse City, MI. The field work showed that BTEX was degraded under denitrifying conditions in conjunction with low oxygen (microaerophilic) levels. However, a suitable control site was not available to test the effects of treatment without nitrate addition. Therefore, further studies were required to ascertain the relative contribution of nitrate to BTEX biodegradation.

The objective of this research was to compare the extent of bioremediation using recharge with and without nitrate addition. Our intent was not to eliminate the other biotic and abiotic processes that might be operating concomitantly with nitrate reduction but to evaluate the benefit of providing nitrate as a supplemental electron acceptor under field conditions. Because this project encompassed the work of several research efforts to provide a thorough site charac-

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S0013-936X(97)01064-X
Published on Web 04/29/1998

terization and performance evaluation of the field project, a complete treatment is beyond the scope of this paper. Instead, we have summarized the results of both the field study and the associated laboratory studies relating to the fate of BTEXTMB (BTEX and trimethylbenzenes) and the role of anaerobic biodegradation. Other information relating to the site characterization (*42*–*46*), changes in microbial populations (*47*), and reduction in sediment toxicity as measured by FETAX (Frog Embryo Teratogenesis Assay, *Xenopus*) (*48*) has been published elsewhere.

Methods

Detailed methods for site characterization, treatability studies, sample analysis, and performance monitoring are given in Appendix A, and supporting data that are referenced in this paper are illustrated in Appendices B and C (see Supporting Information).

Site Description. The field site is located within the Petroleum, Oils, and Lubricants (POL) facility at Eglin Air Force Base, FL, where Air Force personnel had first detected a JP-4 jet fuel leak in an underground pipeline in 1984 (*49*). In 1987, a pilot demonstration project on enhanced in situ biodegradation using hydrogen peroxide was conducted (*50*). Problems with hydrogen peroxide stability in the anaerobic aquifer were encountered, which resulted in a loss of infiltration capacity and reduced oxygen delivery to the subsurface (*51*). The current study was conducted to determine whether anaerobic biodegradation using nitrate as an electron acceptor could be successfully used to continue remediation without loss of infiltration capacity.

Site Characteristics. The treatment area for this study encompassed the area affected by the previous hydrogen peroxide study (Appendix C, Figure 1). The shallow, sandy aquifer is fairly low in TOC and is slightly acidic, especially in the areas of fuel contamination (Table 1). Nitrogen is available as organic nitrogen rather than ammonium or nitrate nitrogen. Areal distribution of the weathered fuel relative to the placement of the treatment cells is shown in Figure 1. This initial site characterization revealed that the residual contamination was distributed 1–2 m below ground surface and the water table varied from 1.0 to 1.2 m below ground surface. The weathered material was depleted in benzene and toluene relative to fresh JP-4 (*42*); however, the fuel-contaminated aquifer was still toxic as compared to background core samples, based on the FETAX assay (*48*). Aqueous nitrate levels were generally less than 0.1 mg/L NO_3-N and nitrite was less than 0.05 mg/L NO_2-N (Appendix B, Table 1). In general, there was a large, viable, and active microbial population, and selected alkylbenzenes could be degraded under denitrifying conditions in corresponding microcosm studies prepared with the aquifer material (*46*).

Field Test Design and Operation. Results from the initial site characterization predicted that surface application would be an effective delivery system (*44*) but that recirculation of recharge water would plug the aquifer due to colloidal material (*45*). On the basis of this, two 30 m × 30 m treatment cells were delineated for treatment. One cell received groundwater recharge amended to yield 10 mg/L of NO_3-N (nitrate cell), and the other received no amendments (control cell). The treatment cells were located downgradient of the original fuel spill area (Figure 1). A raised berm overlying a shallow plastic barrier extending 0.7–1.4 m into the subsurface separated the two cells. There was no other surface or subsurface construction for hydraulic containment. Each cell contained five sprinklers adjusted to cover the cell interior area. These were operated continuously at 42 L min^{-1} $cell^{-1}$ to produce a recharge rate of about 6 cm/day. The recharge water was obtained from the Floridan Aquifer through the drinking water network for that part of the Base. The water had a pH of 7.6 and contained approximately 7 mg/L chloride, 9 mg/L sulfate, 0.1 mg/L NO_3-N, 0.3 mg/L TOC, less than 0.5 mg/L bromide, and less than 0.05 mg/L each of NO_2-N, NH_4-N, PO_4-P, iron, and manganese. BTEXTMB and JP-4 were not detected. Because the water had been chlorinated and still contained 1.8 mg/L chlorine as residual, it was routed through a carbon column to remove chlorine prior to use as recharge.

TABLE 1. Characteristics of Contaminated Aquifer Core Materials Representative of Pre-Test Conditions[a]

Physical Characteristics[b]

parameter	units	sample location EPA1	EPA2
coarse sand (0.5–>2.0 mm)	wt %	32.2 ± 5.0	27.5 ± 6.7
medium sand (0.25–0.50 mm)	wt %	50.2 ± 3.1	53.3 ± 1.6
fine sand (0.05–0.25 mm)	wt %	16.5 ± 2.8	18.8 ± 7.4
silt and clay (<0.05 mm)	wt %	1.2 ± 0.7	0.5 ± 0.3

Nutrient Status[c]

parameter	units	sample location 80EB	80KB
pH	pH units	5.88 ± 0.99	6.49 ± 0.71
ammonium-nitrogen	mg/kg	<0.50	<0.50
nitrate-nitrogen	mg/kg	<0.50	<0.50
nitrite-nitrogen	mg/kg	<0.50	0.8 ± 0.2
total Kjeldahl nitrogen	mg/kg	125 ± 44	73 ± 19
orthophosphate	mg/kg	<0.50	<0.50
total phosphate	mg/kg	30 ± 17	24 ± 23
total organic carbon	wt %	0.07 ± 0.06	0.02 ± 0.02
BTEXTMB	mg/kg	11.2 ± 17.2	<0.001
JP-4	mg/kg	1380 ± 311	<10

Contaminant Distribution[d]

parameter	units	sample location fresh JP-4	cores
alkanes	wt %	58.6 ± 16.8	63.9 ± 3.3
aromatics	wt %	16.8 ± 1.3	13.8 ± 2.3
cycloalkanes	wt %	18.2 ± 1.0	13.8 ± 2.7
alkenes	wt %	1.4 ± 0.7	1.2 ± 0.9
PNAs	wt %	4.1 ± 0.7	5.2 ± 2.7
other	wt %	0.9 ± 0.0	2.1 ± 1.1

[a] Values represent means with standard deviation. [b] Individual cores from 0.3–3.4 m below ground surface, 6–7 depth intervals per location. [c] Individual cores from 0.9–2.1 m below ground surface, three depth intervals per location. 80EB and 80K represent contaminated and uncontaminated locations, respectively. [d] Two replicate samples of JP-4. Cores represent 13 core locations using most contaminated depth interval in each depth profile.

Recharge water and groundwater quality were monitored during system operation using both conventional and cluster monitoring wells. For each cell, a fully penetrating well and a cluster well were placed in the center and at one of the edges (Figure 2). The fully penetrating wells were constructed of 5-cm PVC and screened 0.3–3.4 m below ground surface. The cluster wells consisted of five individual wells per cluster and were installed separately, adjacent to the fully penetrating wells. Each cluster well was constructed of 0.6-mm polypropylene tubing with a 6.4-cm 80-mesh steel screen. The wells were installed 1.2, 1.5, 2.0, 2.6, and 3.4 m below ground surface for each cluster location. A larger, less discrete cluster consisting of three 5-cm PVC wells was designated EPA5A-C and installed downgradient of the nitrate cell (Figure 2). These were screened at 0.3–3.4, 3.4–6.4, and 6.4–9.4 m for EPA5A, EPA5B, and EPA5C, respectively. This well cluster was installed primarily to determine whether nitrate was escaping from the system. For routine monitoring, the recharge waters, wells EPA1–4, EPA5A–C, and the cluster wells were

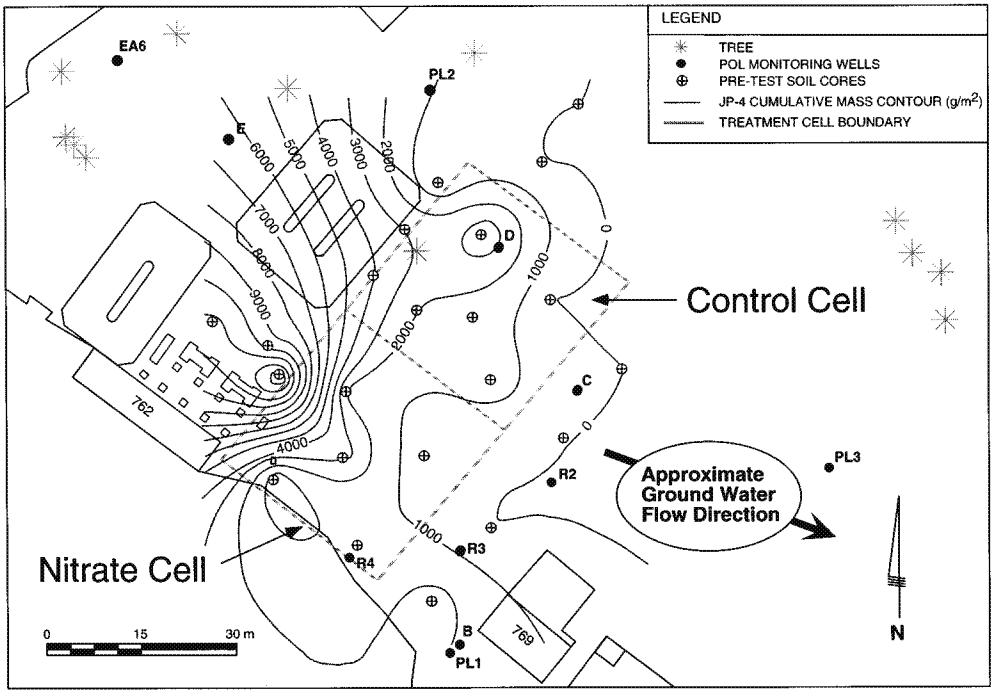

FIGURE 1. Location of POL monitoring wells, pretest core samples, and JP-4 cumulative mass distribution resulting from core analyses. Also shown are the treatment cell boundaries for the pilot demonstration project.

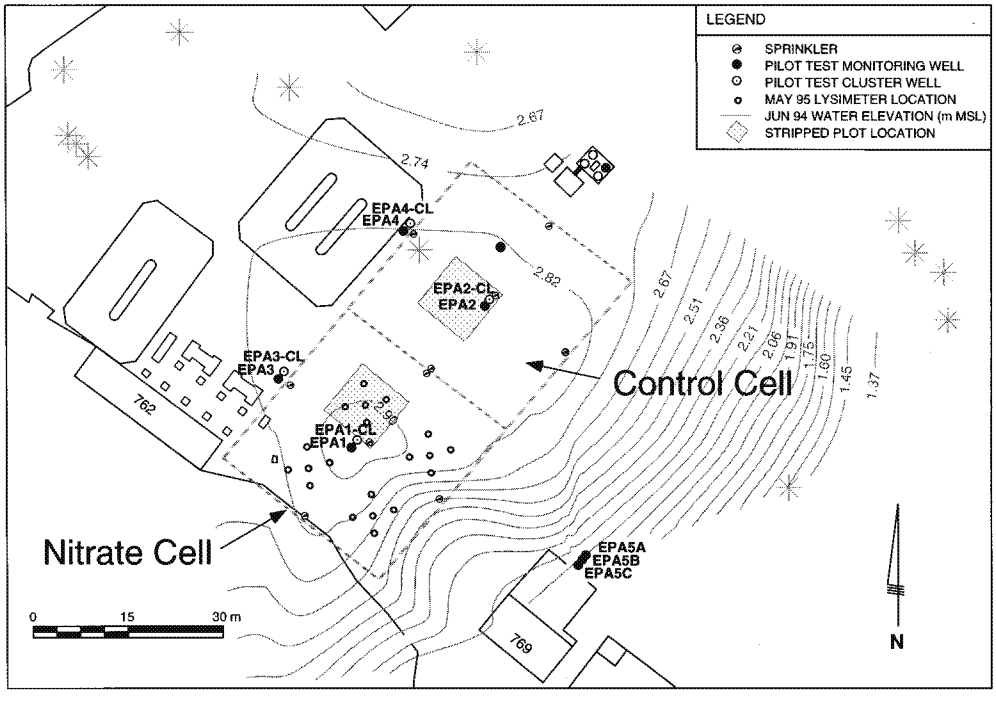

FIGURE 2. Site map schematic showing locations of sprinklers, monitoring wells, and cluster wells installed for pilot project. Also shown are June 1994 water elevation contours during operation, locations of stripped plots installed November 1994, and locations of lysimeters installed May 1995.

sampled semiweekly for the first month and then semimonthly for the duration of the project.

Operation began April 7, 1994, and nitrate levels were increased to 15−20 mg/L NO_3-N on July 15, 1994. Short-term tracer experiments were conducted April 7−21 and June 10−24, 1994, using sodium bromide for the nitrate cell and sodium chloride for the control cell. An interim performance evaluation was conducted August 19−30, 1994. A 9 m × 9 m plot inside each cell was then stripped of vegetative cover on November 14−16, 1994, and covered with weed barrier to enhance nitrate transfer within the nitrate cell (Figure 2). A final performance evaluation was conducted May 13−30, 1995, and the pilot project was discontinued. A final round of water samples was collected May 1996 to evaluate long-term performance.

Performance Evaluations. In addition to routine monitoring for water quality, the two performance evaluations were conducted to provide data on the aquifer sediments as well as more extensive water quality information. As with the initial site characterization, core samples were again obtained for contaminant distribution, microcosm studies, microbial characterization, and toxicological evaluation, and

water samples were obtained from other POL wells and from points adjacent to core locations using the Geoprobe (Appendix A). In addition, for the final performance evaluation, water samples were obtained from 20 lysimeters installed 0.5 m below ground surface at various locations within the nitrate cell and monitored for nitrate, nitrite, ammonium, and TOC (Figure 2). Microcosm tests were conducted with the post-test cores from both cells to evaluate removal of BTEXTMB under denitrifying and iron-reducing conditions.

Results and Discussion

Operational Summary. Operation of the sprinkler system resulted in a water table mound that was maintained throughout the demonstration period (Figure 2), even though the absolute water table varied by more than 1.2 m due to periodic and heavy rainfall events (Appendix C, Figure 2). Results from the initial short-term tracer study, conducted at the start of operation to evaluate water movement when the vadose zone was initially low in water content, showed that recharge had penetrated to the deepest cluster well in the center of each treatment cell (*42*). Background chloride levels were generally low (3−5 mg/L) for all cluster wells at the site but gradually rose to recharge levels (8−10 mg/L) during the study. This even occurred after about 6 months in the deeper cluster wells at the edges of the treatment cells (Appendix C, Figure 3) and in the two shallow downgradient cluster wells EPA5A and EPA5B (Appendix C, Figure 4). This indicates that most of the aquifer within the treatment area had been cleared of the native groundwater. Altogether, the treatment cells received approximately 9000 and 27 000 m^3 of recharge prior to the interim and final performance evaluations, respectively (Appendix B, Table 3). For the nitrate cell, this corresponded to the addition of approximately 90 and 390 kg of NO_3-N during these respective time periods.

Water Quality. The lysimeter data from the interim performance evaluation had shown that most of the applied nitrate was being transported to below the root zone at two of the five locations sampled within the nitrate cell, and microcosm tests with radiolabels confirmed that toluene and *m*-xylene could be mineralized under denitrifying conditions using core samples from one of these locations (*42*). However, the lysimeter data showed that nitrate penetration through the rhizosphere was not uniform, making it difficult to estimate the total mass of nitrate being delivered to the contaminated zone. To address this problem, part of each test cell was stripped of the vegetative cover to facilitate nitrate transport in the nitrate cell and to provide a corresponding control in the control cell. These stripped plots remained essentially vegetation-free for the duration of the study, and the applied recharge permeated quickly and did not pond on the surface. Lysimeter data from the final performance evaluation showed that higher concentrations of nitrate were generally found in water beneath the stripped plot as compared to other areas of the cell, and correspondingly, this groundwater was also generally lower in TOC (Figure 3). These data provide good evidence that initial operation of the pilot cells without removal of vegetation did not allow adequate transfer of electron acceptor to the contaminated zone. However, it is unknown whether nitrate consumption was primarily due to denitrification processes based on decay of vegetative growth or to nitrate-nitrogen assimilation by the vegetation. It is also possible that nitrate transport was enhanced outside of the stripped plots as well during the colder winter months when less vegetative growth and decay would be expected. Regardless, these data show a higher rate of nitrate transport in an area of the nitrate cell that was more highly contaminated (Figure 1).

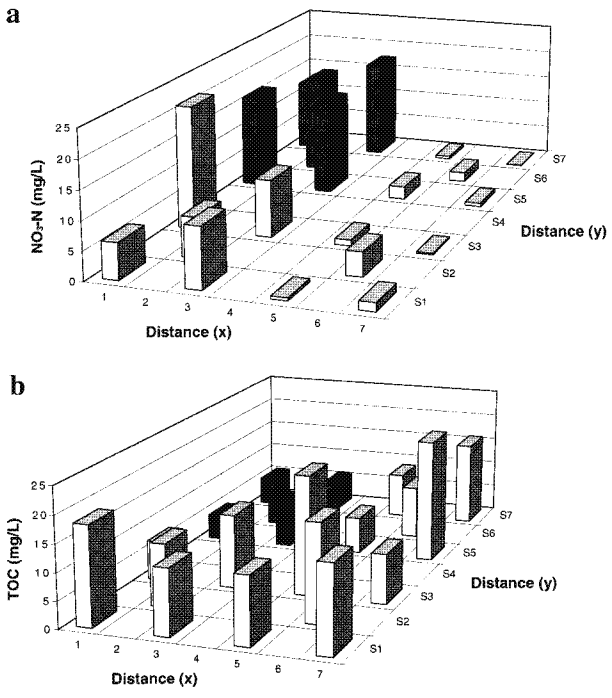

FIGURE 3. Concentrations of (a) nitrate-nitrogen and (b) total organic carbon in nitrate cell lysimeters. Shaded bars represent concentrations beneath stripped plot.

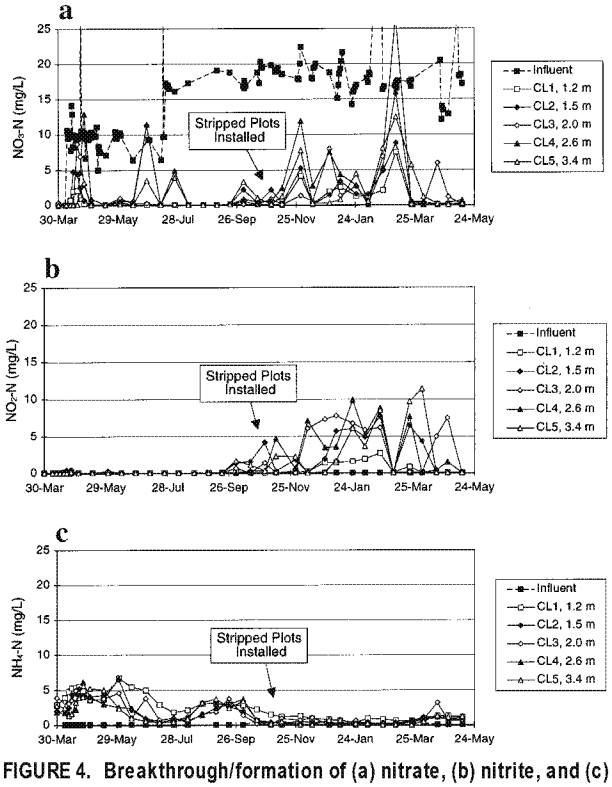

FIGURE 4. Breakthrough/formation of (a) nitrate, (b) nitrite, and (c) ammonium in cluster wells located at center of nitrate cell.

Nitrate concentrations also increased in the EPA1 cluster wells, located in the center of the nitrate cell, following installation of the stripped plots (Figure 4a), and there was a corresponding increase in nitrite concentrations as well (Figure 4b). Corresponding data for all four sets of cluster wells are illustrated in Appendix C, Figures 8−10. Ammonium nitrogen concentrations generally increased in the EPA1 cluster wells during the period of active nitrate removal prior to stripped plot installation (Figure 4c). This indicates the

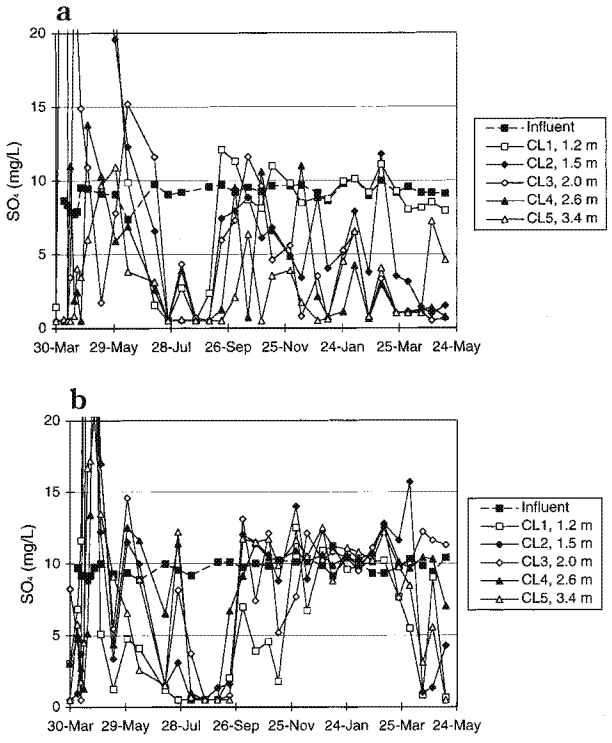

FIGURE 5. Breakthrough of sulfate in cluster wells located at (a) center of control cell and (b) center of nitrate cell.

potential for dissimilatory nitrate reduction to ammonium, which is consistent with the hypothesis that the fuel-contaminated aquifer was depleted in electron acceptors. Further evidence of dissimilatory nitrate reduction to ammonium is provided by post-test microcosm studies, demonstrating ^{15}N ammonium production from ^{15}N nitrate utilization (Appendix C, Figure 13). Because dissimilatory nitrate reduction to ammonium would more likely be expected under electron acceptor-limited conditions (*17*), these data provide supporting evidence that nitrate was being used as an electron acceptor in the contaminated subsurface within the nitrate cell. The fact that nitrate transport was enhanced and ammonium concentrations decreased after the stripped plots were installed also supports this hypothesis. Aqueous BTEXTMB levels in the EPA1 cluster wells had generally decreased prior to installation of the stripped plots, although there were still high concentrations observed in the shallowest cluster well, possibly due to residual saturation at this location (Appendix C, Figure 5). Periodic concentration spikes were observed at other levels as well. Dissolved oxygen was rarely detected in either cell (Appendix C, Figure 6). Collectively, these data indicate that nitrate may have been the primary electron acceptor for that area of the nitrate cell encompassing or adjacent to the stripped plot.

Interestingly, sulfate concentrations in the nitrate cell EPA1 cluster wells began to approach those in the recharge water, following an initial leaching of sulfate from the upper soil layers (Figure 5). In contrast, sulfate concentrations generally decreased in the control cell cluster wells following installation of the stripped plots, although the results were much more variable. In addition, thiosulfate was generally detected in these cluster wells as compared to those in the nitrate cell (Appendix C, Figure 12). These data indicate that sulfate may have been the primary electron acceptor for that area of the control cell adjacent to the stripped plot. Methane and soluble manganese were not routinely monitored, and soluble iron concentrations were similar for both treatment cells (Appendix C, Figure 7). Therefore, other anaerobic processes such as iron reduction, manganese reduction, and methanogenesis may also have contributed, but their relative effects are unknown. Analysis of cluster well samples taken during the interim performance evaluation showed higher levels of organic acid intermediates in cluster wells from the nitrate cell as compared to those from the control cell (Appendix B, Table 4). The presence of organic acid intermediates indicates that biodegradation of fuel hydrocarbons was occurring in the nitrate cell. It is possible that other anaerobic processes were also occurring in the control cell, but that similar intermediates were either not produced or were metabolized more quickly than the parent compounds.

Core Data. Mass estimates, based on core analyses, were made for various contaminant groups in both treatment cells during the study. Remediation was minimal in both cells for the first 4 months of the study, based on core data from the initial site characterization and the interim performance evaluation (Appendix B, Table 5). This was surprising, given the other evidence that biodegradation was occurring during this time, and may represent an artifact that resulted because of the following: (a) mid-test core locations were not selected adjacent to pre-test core locations, (b) site heterogeneities precluded even distribution of recharge, and (c) the extent of nitrate uptake within the rhizosphere was highly variable prior to installation of the stripped plots. In contrast, the post-test cores were obtained from locations adjacent to those in the interim performance evaluation to minimize the effects of site heterogeneity. This analysis therefore focuses on mass estimates calculated for the interim versus the final performance evaluation, and the data show significant mass reduction of BTEXTMB in both treatment cells (Figure 6). Mid-test mass estimates were 160 kg of BTEXTMB and 5870 kg of JP-4 in the nitrate cell and 33 kg of BTEXTMB and 1750 kg of JP-4 in the control cell. On the basis of core data from the final performance evaluations, BTEXTMB was reduced by 66 ± 1% in both treatment cells, equivalent to a mass loss of 106 and 21 kg in the nitrate cell and control cell, respectively. In contrast, JP-4 decreased by 37% (2170 kg) in the nitrate cell and increased by 11% (210 kg) in the control cell.

On the basis of the BTEXTMB information, both treatment cells were remediated to the same extent, although the nitrate cell contained five times more contaminants on a weight basis. It is not possible to differentiate between remediation by biological activity versus soil washing based on these data alone. Regardless, this mass reduction led to a corresponding reduction in aqueous BTEXTMB concentrations both within and downgradient of the treatment cells. One year after the study was completed and the groundwater was allowed to reattain equilibrium, the cluster wells in the centers of both treatment plots still showed the effects of the pilot study, with an average reduction in aqueous BTEXTMB concentrations of 80 ± 21% and 87 ± 12% in the nitrate and control cells, respectively, as compared to cluster well concentrations at the beginning of the study (Appendix B, Table 6). Downgradient wells showed a corresponding aqueous BTEXTMB concentration reduction of 72 ± 34%, which correlates well with the observed mass reductions in the treatment cells.

To better assess the effects of soil washing versus bioremediation, three additional locations within each of the stripped plots had been sampled during the final performance evaluation to determine whether removal of the vegetative cover enhanced bioremediation. In the nitrate cell, BTEXTMB mass removal was 96 ± 4% within the stripped plot area as compared to 65 ± 17% immediately outside of it. Comparison of BTEXTMB removal within (41 ± 42%) and outside (68 ± 28%) of the control cell stripped plot was inconclusive due to the high variability associated with these data. It is doubtful that the enhanced removals observed

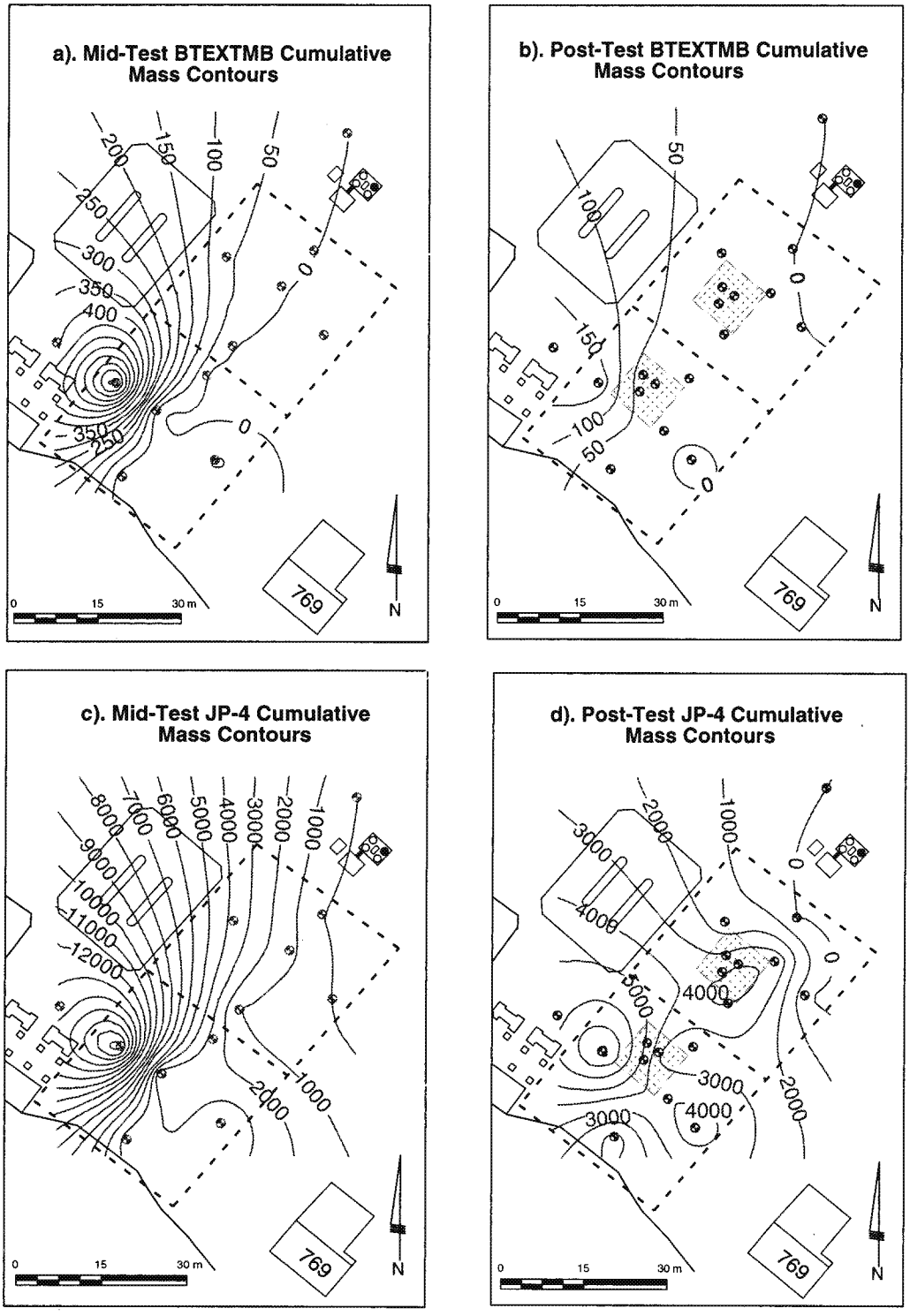

FIGURE 6. Mass removal of BTEXTMB and JP-4 based on core analyses. Shown are cumulative mass contours (g/m^2) of BTEXTMB based on cores taken during (a) interim and (b) final performance evaluation and corresponding mass contours of JP-4 during (c) interim and (d) final performance evaluation.

within the stripped plots are due to increased water transport through the contaminated region, since the rate of infiltration is substantially higher than the rate of evaporation in this humid climate. A more plausible explanation is that the installation of the stripped plots significantly reduced the amount of vegetative organic matter competing with the fuel hydrocarbons for the available electron acceptors, including nitrate in the nitrate cell and perhaps sulfate in the control cell.

Microcosm Tests. To determine whether other anaerobic processes could have contributed to BTEXTMB removal in both the nitrate and control cells, microcosms were prepared with core samples aseptically obtained from three depths at locations within the center of each cell. In those cores that exhibited BTEXTMB removal, biodegradation of BTEXTMB occurred predominantly under nitrate- and/or iron-reducing conditions within the first 30 days (Figure 7), but not under sulfate-reducing or methanogenic conditions (data not

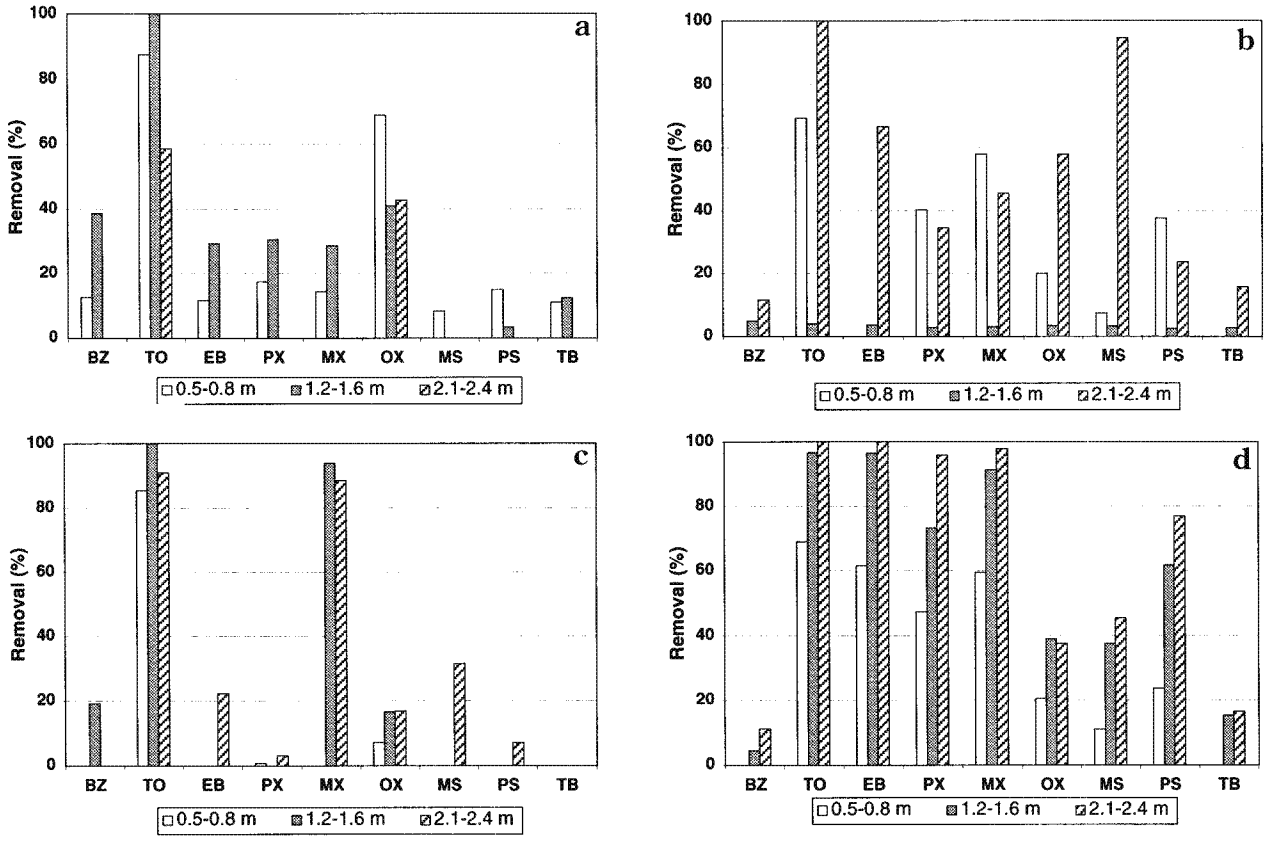

FIGURE 7. Removal of BTEXTMB isomers from (a and c) control cell and (b and d) nitrate cell microcosms, with either nitrate or iron addition. Removals are shown for the first 30 days of incubation. Mean of three replicates per set, corrected for killed controls.

shown). In the nitrate cell cores, there was significant BTEXTMB removal at each depth under denitrifying conditions and at two levels under iron-reducing conditions (Figure 7). In contrast, BTEXTMB removal under denitrifying conditions occurred to a much less extent in the control cell. It is especially interesting that BTEXTMB removal was generally limited to iron-reducing conditions in the upper layer of the control cell. Additional microcosm tests with radiolabeled *m*-xylene confirmed that mineralization of this compound under denitrifying conditions did not occur in this layer even after 200 days, even though mineralization was observed under iron-reducing, sulfate-reducing, and methanogenic conditions (Appendix C, Figure 14). BTEXTMB biodegradation occurred under both denitrifying and iron-reducing conditions in the upper layer of the nitrate cell (Figure 7). This depth is just below the level sampled by the lysimeters and provides supporting evidence that nitrate which was transported to this interval in the nitrate cell was probably used for BTEXTMB biodegradation.

Bioremediation versus Soil Washing. Because the recharge water was not captured and recirculated, it is not possible to quantitate the relative effects of enhanced bioremediation versus soil washing in this pilot demonstration project. Nevertheless, the example data support the role of bioremediation as a major component in the observed remediation. Microcosm data show that biodegradation of BTEXTMB in this aquifer could occur under different electron-acceptor conditions, and the establishment of different active microbial populations within the contaminated intervals in each of the treatment cells would imply that significant contaminant removal occurred through microbial processes. Furthermore, had soil washing played a major role, most of the BTEXTMB mass removal should have occurred during the first phase of the study, and the core mass data do not support this (Appendix B, Table 5). Additional microcosm tests showed a definite enhancement in BTEXTMB biodegradation under denitrifying conditions downgradient of the nitrate cell (Appendix C, Figure 15). These populations were most likely active during remediation, since elevated nitrate concentrations in the contaminated intervals below the rhizosphere of the nitrate cell were not detected in any downgradient wells (Appendix B, Table 1). Column tests conducted with the post-test aquifer material and operated under similar hydraulic regimes showed that 96% of the BTEX mass and 78% of the BTEXTMB mass that were mobilized were biologically degraded within the columns (*52*). Hence, much of the BTEXTMB initially mobilized through soil washing may have been degraded under denitrifying conditions during transport away from the treatment area. In addition, modeling efforts currently underway indicate that soil washing alone was insufficient to account for removal of a test labile trimethylbenzene isomer within the nitrate cell (*53*). Collectively, these data indicate that most of the BTEXTMB mobilized through soil washing was biologically metabolized.

Application of Mixed Electron Acceptor Processes. Regardless of the specific anaerobic process or processes involved, an important aspect of this research is that simple recirculation of recharge, without added amendments, can still promote bioremediation in fuel-contaminated aquifers as long as endogenous electron acceptors are present. An advantage of nitrate-amended recharge in this case may have been to increase soil pH and to facilitate the formation and transport of ammonium throughout the aquifer, enhancing microbial activity in general (Appendix B, Table 7). In our study, we cannot assess the relative benefits of indigenous electron acceptors in the recharge versus the mobilization of electron acceptors in the vadose zone. Despite this, the field and laboratory data indicate that it may be advantageous to utilize this approach to promote a variety of anaerobic

processes rather than to try to establish one type of reaction, such as denitrification. In heterogeneous environments, more than one microenvironment conducive to selective reactions is likely to exist, and establishment of these separate microenvironments should be encouraged rather than controlled. These different environments would encourage biodegradation of other compounds that are generally recalcitrant under denitrifying conditions, such as benzene. Although benzene was not a significant contaminant at this particular site, it can be degraded under iron-reducing, sulfate-reducing, and methanogenic conditions (*54*−*56*), and these processes occurred in both treatment cells.

At this site, most of the contamination was not in the vadose zone. In addition, the normal depth to water varied from 1.0 to 1.2 m below ground surface, and the contaminated zone generally extended from 1 to 2 m below ground surface. Although these conditions do not favor either bioventing (*13*) or biosparging (*14*), these aerobic treatment methods should be considered as well for different hydrologic settings (*12*). In situ aerobic remediation with hydrogen peroxide was problematic at this field site (*51*), but additional comparative studies are needed in comparing air mobilization strategies to mixed electron acceptor processes for bioremediation of anaerobic aquifers. Field research is also required to optimize the use of mixed electron acceptor treatment regimes and determine whether this strategy should be considered for sites where contaminant levels are too high to allow natural attenuation processes to proceed at rates sufficient to protect downgradient receptors.

Acknowledgments

The authors would like to acknowledge the research efforts of Howard Sweed, Phil Bedient, Michele Thomas, Herb Ward, and Mark Wiesner of Rice University and Jack Bantle of Oklahoma State University, whose collaborative work on the field project provided the basis for much of the interpretation. The authors would also like to thank the following groups and individuals for their support and cooperation: (a) Frank Beck et al., RSKERC, for field support in drilling and well construction; (b) Stephen Williams, Environmental Restoration Program, Eglin AFB, for providing oversight and coordinating access to the field site; (c) Guy Willis, EA Engineering, for providing monitoring and sampling services; (d) ManTech Environmental Research Services Corporation, for providing analytical support; and (e) Computer Data Systems Inc, for graphics, mapping, contouring, and modeling services. Although the research described in this paper has been funded wholly or in part by the U.S. Environmental Protection Agency and the U.S. Air Force (MIPR N92-65, AL/EQ-OL, Environmental Quality Directorate, Armstrong Laboratory, Tyndall AFB), it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

Supporting Information Available

Three appendices giving the detailed methods (Appendix A), tables (Appendix B), and figures (Appendix C) (55 pp) will appear following these pages, in the microfilm edition of this volume of the journal. Photocopies of the Supporting Information from this paper or microfiche (105 × 148 mm, 24× reduction, negatives) may be obtained from Microforms Office, American Chemical Society, 1155 16th St. NW, Washington, DC 20036. Full bibliographic citation (journal, title of article, names of authors, inclusive pagination, volume number, and issue number) and prepayment, check or money order for $90.00 for photocopy ($92.00 foreign) or $12.00 for microfiche ($13.00 foreign), are required. Canadian residents should add 4% GST. Supporting Information is also available via the World Wide Web at URL http://www.chemcenter.org. Users should select Electronic Publications and then Environmental Science and Technology under Electronic Editions. Detailed instructions for using this service, along with a description of the file formts, are availableat this site. To download the Supporting Information, enter the journal subscription number from your mailing label. For additional information on electronic access, send electronic mail to si-help@acs.org or phone (202)872-6333.

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Received for review December 9, 1997. Revised manuscript received March 20, 1998. Accepted March 25, 1998.

ES971064L